Research Article

Cholesterol lowering effect of Citrus paradisi (Grape fruit) peel extract in hypercholestrolemic patients in an urban community:A randomized control trial.

Maaz Ahmad[1], Hamna Ahmad[2], Mussab Ahmad[3], Tehreem Munir[4]

[1]Professor of Community Medicine, Rashid Latif Medical College, Lahore, Former Dean Faculty of Preventive Medicine, KEMU, Former Dean Institute of Public Health, Lahore; [2]Lecturer University of Lahore; [3]Medical Officer, Mayo Hospital, Lahore; [4]Mayo Hospital, Lahore

Abstract

Background: Cardiovascular diseases (CVDs) account for 31% of all deaths worldwide and 80%of all CVD deaths are due to heart attacks and strokes . Atherosclerosis is the most common cause of CVDs. Hypercholesterolemia is the major cause of atherosclerosis. Based on a literature search, no studies have been published concerning the cholesterol lowering effects of Citrus paradisi (Grape fruit) peel extract in human beings so far.

Objective: To assess cholesterol lowering effect of Citrus paradisi peel extract (CPE) in hypercholestrolemic patients in an urban community at Lahore

Material & Methods: A double blind, placebo-controlled, multicentred, randomized control trial was conducted in Lahore urban community for a period of 6 weeks with the help of family physicians from Sep.2016 to March 2017. The study included randomly selected 200 hypercholesterolemic male patients of age group 40 to 70 years, non-smokers ,not in habit of doing exercise daily, and who consented to the trial. Selected patients were placed in 2 groups i.e trial(CPE) group and placebo group. In trial group, patients were treated with CPE (10 mcg in 2 divided doses) for 6 weeks. In placebo group similar looking preparation was given to the patients for the same period. Evaluation of lipid profile was the outcome measure.

Results: There was significant reduction in the levels of cholesterol, triglycerides and low density lipoprotein whereas significant improvement in high density lipoprotein level in the CPE group as compared with placebo group ($p<0.001$). Regarding blood profile , no adverse event was observed.

Conclusion: C.paradisi extract(CPE) significantly reduced cholesterol level in patients with hypercholesterolemia .

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Corresponding Author | Prof. Maaz Ahmad, Professor of Community Medicine, Rashid Latif Medical College, Lahore, Former Dean Faculty of Preventive Medicine, KEMU, Former Dean Institute of Public Health, Lahore

E-mail: profmaaz@gmail.com

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Introduction

Hyperlipidemia is a diverse group of disorders categorized by the increased levels of lipids and lipoproteins in the blood e.g., cholesterol, triglycerides, low density lipoprotein (LDL), and VLDL (very low-density lipoprotein), which eventually leads to atherosclerosis. Atherosclerosis is a disease in which plaque builds up inside the arteries. It is commonly known that arteries are blood vessels responsible for carrying oxygen-rich blood to your heart, lungs and other parts of your body. Plaque is made up of cholesterol, fat, calcium, and other substances which are found in the blood. This

deposition makes the arteries narrow and hard causing reduced blood flow to vital organs, especially the heart and brain which may result into coronary heart disease (CHD) or stroke.[1] It is estimated that, from 1980 to 2000, reduction in total cholesterol accounted for a 33% decrease in coronary heart disease (CHD) deaths in the United States. In other developed countries, similar decreases in CHD deaths (ranging from 19%–46%) have been attributed to reduction in total cholesterol.[2]

Many herbs have been found effective in reducing cholesterol level in hypercholesterolemic patients. Citrus paradisi (C.paradisi) is one of such novel fruits rectifying the problem of hypercholesterolemia. People developed an interest in grapefruit because of its lipid-lowering abilities, which may be due to the high pectin contents of grapefruit, a soluble fiber that lowers blood cholesterol.[3]

Grapefruit (Citrus paradise) has been investigated in many studies. Drinking grapefruit juice might be cardio-protective through increasing total antioxidant status and decreasing lipid peroxidation independent of any of the cardiovascular risk markers measured in the study. The scientists found that feeding some patients the equivalent of one grapefruit daily significantly reduced level of cholesterol when compared to patients who did not eat grapefruit.[4] In a study flavonones contained in C.paradisi protected from arterial stiffness in women.[5] An animal study was conducted to determine the hypolipidemic effect of C. paradisi in rats receiving diet rich in cholesterol and it showed a highly significant fall in cholesterol, low density lipoprotein(LDL), very low density proteins(VLDL) and triglycerides(TGs), however high density lipoproteins(HDL) level was significantly elevated.[6] Its anti-oxidant and anti-inflammatory effects playing a role in the inhibition of atherogenesis was described in a study conducted by Rafiq A Khan[7]. To evaluate the evidence for or against the effectiveness of grapefruits (Citrus paradisi) on body weight, blood pressure, and lipid profile was conducted and it indicated the protective role of Citrus paradise[8]. Lipid lowering effect of extract of C.paradisi was revealed in a study and results showed significant dose related lowering effects of the extract on lipid parameters. [9]

Grape fruit is eaten by many persons who might be taking cholesterol lowering medicines like simvastatin. So it is a warning for simvastatin users that simultaneous intake of grapefruits on regular basis might be producing harmful effects. One study showed enhanced cholesterol-lowering effect because of boosting simvastatin as it considerably increased the plasma concentrations of simvastatin and simvastatin acid in the body.[10] Boosting effects of Simvastatin and simvastatin acid was also observed in other studies.[11,12]

In a cross-over study, consumption of grapefruit juice decreased LDL-cholesterol by 6%.[13] A study indicated the beneficial effects of grape fruit for hyperlipidemic, especially hypertriglyceridemic, patients suffering from coronary atherosclerosis.[14] C.paradisi is rich in lycopene which is strong anti-oxidant and found associated with decreasing oxidation of low density lipoproteins (LDL) thus decreasing the risk of atherosclerosis and in turn CHD.[15] Cholesterol lowering effect of C.paradisi has been much studied and the role of lycopene has been found significantly effective.[16] This antioxidant property protects the cells from DNA damage and reduces the oxidative damage to lipids and proteins.[17,18]

To assess the effectiveness of Citrus paradisi extract (CPE) in treating hypercholesterolemia this study was designed ..

Methods

500 grams peelings of C.paradisi fruit were dried and powdered resulting into 100 grams powder which was processed through anaerobic sublimation A 10 grams residue was obtained. An ethanolic-aqueous extraction of the obtained residue was performed. The resultant extract was diluted to make 10 microgram extract in one teaspoonfull.[19]

Multi-stage sampling method was applied to select an urban community to carry out this research. Lahore urban community comprised of 10 towns. One town i.e. Allama Iqbal Town , was selected through random sampling. Out of 19 union councils one was randomly selected. A double-blind, randomized, placebo-controlled, multicenter research trial (RCT) was conducted in that selected union council for a period of 6 weeks (in a period from Sep.2016 to March 2017) with the help of randomly selected leading family physicians practicing in the above mentioned union

council. Thus randomly selected 10(Ten) out of 50 (Fifty) family physicians participated in the study. Sample size for this trial was estimated through this formula:

$$n_e = n_c = \{(Z_{\alpha/2}+Z_{\beta})^2\delta^2(\lambda+1)/\lambda\}/(\mu_c-u_e)^2$$

In this study, the ratio (λ) of C. paradisi group to placebo group was 1:1 and keeping power of 80% (1 - β) at significance level of 5% (α), a sample size of $n_e = n_c = 100$ patients per treatment group was taken. In this study, sample was 200 patients(Total cholesterol levels more than 200 mg/dL) .Out of this sample 100 patients were placed in trial group and 100 patients in placebo group.

The patients included in the trial were of age group 40 to 70 years, non-smokers and not in habit of doing exercise and who had not received lipid-lowering medications for at least 6 months prior to enrollment in this study. Any case suffering from diabetes mellitus, acute or chronic hepatic disease, renal impairment, cardiac disease, severe organic or psychiatric illness was excluded. Pregnant and lactating mothers were excluded from this trial. Any case using any sort of other herbal medicines, with the complaints of abrupt weight loss ,passing black stool or refusing to participate was also excluded.

The selected family physicians enrolled 15-25 hypercholesterolemic patients in the trial in a randomized way after taking written consent for participation in the trial and with the help of proforma they recorded their data. Out of hypercholesterolemic patients attending their clinics ,it was decided through random sampling about the selection of first/second/ third or any order patient with hypercholesterolemia. If the selected patient was not fulfilling the laid down criteria then the next patient fulfilling the same criteria was selected. They placed hypercholesterolemic patients in either C.paradisi group or Control group according to the order decided, so equal number of participants in C.paradisi group and Control group were enrolled.

As the study was double blind, so the researchers (Family physicians) were neither aware of the actual extract and placebo nor the patients as both samples of extract and placebo were of same color and were supplied in bottles of same size, shape and color. Only the code numbers written on the bottles were different.

For 6 weeks, both groups were advised to eat a low-calorie, low-fat diet and also advised not to have any bazaar cooked food. Cases were randomly given either C.paradisi extract (0.6 microgram in 10 drops twice a day) or placebo (10 drops of placebo twice a day) for a period of 6 weeks.

Lipid profile was evaluated at baseline and then after 6 weeks. Routine tests of blood (complete blood count, liver profile, renal profile, blood sugar and lipid profile) were performed at the start and after completion of trial to know any change in these parameters due to use of C.paradisi extract or placebo. Before the start of trial, both groups (CPE and Control) were compared by using Independent sample 't' Test. Changes in the blood profile before and after the trial were analyzed through the application of paired 't'Test by using SPSS version 20.

Results

In this trial 200 hypercholesterolemic patients participated. There was no significant age and gender difference in both groups ($p>0.05$). See Table 1

Before the start of trial, both groups (CPE and Control) were evaluated statistically for routine blood parameters by applying Independent sample 't'Test revealing no significant difference.

See Table.2.

At the end of trial, each group was re-evaluated separately for any change in routine blood parameters. This evaluation was done to see all the blood parameters in CPE group and placebo group particularly lipid profile. Applying paired 't' Test, results showed that there was significant reduction in CPE group regarding cholesterol, triglycerides and low density lipoproteins whereas there was found significant rise in high density lipoproteins in the CPE group as compared with the placebo group. So CPE intake markedly reduced hypercholesterolemia. See Table 3.

Table 1: *Demographic Information of Participants*

Parameter	Trial (*n*=100)	Control (*n*=100)	P-value
Age (years)	41.23±4.22	42.34±4.78	0.765
Male (cases)	45	42	0.319
Female (cases)	55	58	

Table 2: *Comparison of Blood Parameters before Intervention in both Groups*

Blood Profile		CPE Group	Placebo Group	p-value S=Significant NS=Not significant
Parametre	Desirable Range	Mean±SD	Mean±SD	Mean±SD
Hemoglobin (Hb)	13-18 gm/dL	14.12±1.41	14.11±1.31	>0.05 (NS)
Red blood cells (RBC)	4.4-5.8 million/ml	4.8±0.27	4.8±0.26	>0.05 (NS)
Total leucocyte count(TLC)	4000-11000/mcL	7845±1587	7845±1577	>0.05 (NS)
Neutrophils	2.0–7.0×10^9/L (45-70%)	58.21±1.67	58.31±1.61	>0.05 (NS)
Lymphocytes	1.0–3.0×10^9/L (20-50%)	31.49±1.45	32.50±1.32	>0.05 (NS)
Monocytes	0.2–1.0×10^9/L (2-10%)	3.31±1.27	3.31±1.33	>0.05 (NS)
Eosinophils	0.02–0.5×10^9/L (0-6%)	2.56±.59	2.56±.73	>0.05 (NS)
Platelets	150000-450000 cells/mcL	328104±41876	328104±41501	>0.05 (NS)
Blood sugar random	<140 mg/dL	132.34±21.87	132.34±20.98	>0.05 (NS)
Bilirubin	0.2 to 1.2 mg/dL	.68±.16	.68±.18	>0.05 (NS)
Alanine transaminase (ALT or SGPT).	<50 U/L	28.26±7.59	28.25±7.45	>0.05 (NS)
Aspartate transaminase (AST or SGOT)	<50 U/L	29.98±6.87	30.01±6.88	>0.05 (NS)
Alkaline phosphstase(ALP)	<258 U/L	132.67±12.01	132.69±12.11	>0.05 (NS)
Blood urea	15-43 mg/dL	14.67±4.34	14.98±4.33	>0.05 (NS)
Serum creatinine	0.5-1.5 mg/dL	.743±.08	.743±.08	>0.05 (NS)
Total cholesterol (TC)	<200 mg/dL	235.58±14.87	235.20±14.72	>0.05 (NS)
Triglycerides(TG)	<150 mg/dL	220.23±11.67	220.11±11.57	>0.05 (NS)
High density lipoprotein(HDL)	>45 mg/dL	38.76±6.64	38.75±6.59	>0.05 (NS)
Low density lipoprotein (LDL)	<130mg/dL	152.78±12.34	152.43±12.33	>0.05 (NS)

Table 3: *Comparison of Blood Parameters before and after Trial in each Group(Applying Paired 't' Test)*

		CPE Group			Placebo Group		
		Before	After	p-value	Before	After	p-value
Parametre	Desirable Range	Mean±SD			Mean±SD		
Hb	13-18 gm/dL	14.12±1.41	15.12±1.31	>0.05 (NS)	14.11±1.31	13.67±1.09	>0.05 (NS)
RBC	4.4-5.8 million/ml	4.8±0.27	5.9±0.32	>0.05 (NS)	4.8±0.26	4.8±0.26	>0.05 (NS)
TLC	4000-11000/mcL	7845±1587	7855±1365	>0.05 (NS)	7845±1577	7835±1486	>0.05 (NS)
Neutrophils	2.0–7.0×10^9/L (45-70%)	58.21±1.67	58.26±1.55	>0.05 (NS)	58.31±1.61	60.42±1.71	>0.05 (NS)
Lymphocytes	1.0–3.0×10^9/L (20-50%)	31.49±1.45	32.19±1.29	>0.05 (NS)	32.50±1.32	33.49±1.34	>0.05 (NS)
Monocytes	0.2–1.0×10^9/L (2-10%)	3.31±1.27	3.27±1.31	>0.05 (NS)	3.31±1.33	3.29±1.31	>0.05 (NS)
Eosinophils	0.02–0.5×10^9/L (0-6%)	2.56±.59	2.57±.61	>0.05 (NS)	2.56±.73	2.55±.71	>0.05 (NS)
Platelets	150000-450000 cells/mcL	328104±41876	328112±43512	>0.05 (NS)	328104±41501	328104±38764	>0.05 (NS)
Blood sugar ®	<140 mg/dL	132.34±21.87	127.54±19.76	>0.05 (NS)	132.34±20.98	137.34±21.56	>0.05 (NS)
Bilirubin	0.2 to 1.2 mg/dL	.68±.16	.67±.32	>0.05 (NS)	.68±.18	.71±.04	>0.05 (NS)
ALT (SGPT).	<50 U/L	28.26±7.59	27.54±7.08	>0.05 (NS)	28.25±7.45	29.31±6.97	>0.05 (NS)
AST (SGOT)	<50 U/L	29.98±6.87	28.98±6.05	>0.05 (NS)	30.01±6.88	29.23±6.43	>0.05 (NS)
ALP	<258 U/L	132.67±12.01	131.67±11.08	>0.05 (NS)	132.69±12.11	131.54±11.98	>0.05 (NS)
Blood urea	15-43 mg/dL	14.67±4.34	13.32±3.12	>0.05 (NS)	14.98±4.33	15.21±5.13	>0.05 (NS)
S.Creatinine	0.5-1.5 mg/dL	.743±.08	.730±.06	>0.05 (NS)	.743±.08	.751±.11	>0.05 (NS)
Total Cholesterol	<200 mg/dL	235.58±14.87	198.16.±10.23	<0.05 (S)	235.20±14.72	245.51±15.43	>0.05 (NS)
S.Triglycerides	<150 mg/dL	220.23±11.67	159.11±16.07	<0.05 (S)	220.11±11.57	258.28±22.64	>0.05 (NS)
HDL	>45 mg/dL	38.76±6.64	44.56±6.31	<0.05 (S)	38.75±6.59	37.54±6.34	>0.05 (NS)
LDL	<130 mg/dL	152.78±12.34	121.78±12.34	<0.05 (S)	152.43±12.33	156.32±12.89	>0.05 (NS)

Discussion

Hypercholesterolemia is the main cause of cardiovascular disease (CVD) and cellular cholesterol homeostasis is of vital importance in preventing CVD.[2]

The present study shows that C.paradisi extract given to hypercholesterolemic patients daily in two divided doses of 0.6 microgram each for a period of 6 weeks produced lipid lowering effects as compared to placebo. It was found that this trial significantly decreased cholesterol, triglycerides and low density lipoproteins and at the same time enhanced the level of high density lipoproteins in the experimental group whereas there was no significant change observed in the control group.

Results of the present study have been found supported by many studies conducted in the past.

One study revealed the antihyperlipidemic effect of C.paradisi endorsing the findings of present study.[3] Similar results were obtained in another study in which it was observed that an intake of one grapefruit in a day could help keep heart disease away.[4] This effect was supported by a study which reveals that flavonones contained in C.paradisi protected arterial stiffness.[5] The present study results were verified by an animal study in which C.paradisi juice was given to rats receiving diet rich in cholesterol showed a highly significant fall in cholesterol, low density lipoproteins, and triglycerides, however high density lipoproteins level was significantly elevated[6]. Its antioxidant and anti-inflammatory effects playing a role in the inhibition of atherogenesis supported the results of present study[7]. Present study results were endorsed by the results of a study conducted to evaluate the evidence for or against the effectiveness of grapefruits on body weight, blood pressure, and lipid profile.[8,9]

Simvastatin is a known lipid lowering medicine used widely in the world. Some studies demonstrated the lipid lowering and simvastatin boosting effect of C.paradisi.[10,11,12]

Role of lycopene is very important in lowering the lipids. This carotenoid present in C.paradisi has been found responsible for lowering cholesterol in our study like other studies. Cholesterol lowering effect of C.paradisi has been much studied in the past and the role of lycopene has found significantly effective.[13,14,15] Lipid lowering effect due to lycopene present in C.paradisi was also observed in another study.[16,17,18]

The current study is unique in many aspects. Firstly CPE was used in very minute quantity (in micrograms) as compared with the amount of Citrus paradisi juice given in other studies or its extract by the past researches. Secondly there was no side effect or adverse effect reported. Thirdly for the first time ethanolic-aqueous extract after anaerobic sublimation was used in humans to manage hypercholesterolemia and it might explain the efficacy of the extract prepared in a special way. Lastly it opens the doors for further research for improving human health regarding other parts of citrus paradisi like flowers. leaves or roots.

We evaluated and compared the effects of CPE with placebo and found that in this six-week trial significant reduction in cholesterol was observed in CPE group as compared with placebo. Extensive trials may be required to evaluate the efficacy of CP extract and follow up studies should be conducted to see the sustainability of this new remedy.

This study opens a new horizon to evaluate amazing effects of plants on the human health. There is dire need to conduct more studies to explore the beneficial effects of grapefruit to find some better alternate solutions of managing hypercholesterolemia which is a leading cause of atherosclerosis and in turn high cardiac mortality. There is also need for laboratory experiments to reveal the mechanism of cholesterol lowering effect of C. paradisi extract.

Conclusion

Cholesterol lowering effect of Citrus paradisi peel extract (CPE) in hypercholestrolemic patients in an urban community at Lahore was assessed in this study. It was found that there was significant drop in the levels of blood cholesterol, triglycerides and low density lipoproteins in the trial group whereas the level of high density lipoproteins was significantly raised. In this trial one thing is unique that very little amount of citrus paradisi peel extract revealed amazing results and it may be postulated to be an alternate remedy to reduce hypercholesterolemia.

Take away message will be to explore indigenous resources for effective and economical solutions for public health issues.

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